

Amendments to the Specification:

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 4, line 20 extending to page 5, line 14:

A 2

-In one embodiment, this invention provides a substantially pure nucleic acid which is homologous with a *EcoRI/XhoI* fragment isolated from λ phage clone 44B.1 deposited under ATCC accession No. PTA-3170 [N]. Sequences of this invention are homologous as determined by a sequence alignment score of greater than 200 as calculated by BLASTN 2.1.2. Alternatively, this invention provides a substantially pure nucleic acid which hybridizes to a *EcoRI/XhoI* fragment isolated from λ phage clone 44B.1 deposited under ATCC accession No. PTA-3170 [N] under stringent conditions, wherein stringent conditions comprise 0.5 M NaHPO₄ /1mM EDTA/7% (w/v) SDS at 55°C, preferably 60°C, more preferably 65°C. The nucleic acid may be DNA or RNA, and may be produced by recombinant methods. The nucleic acid is preferably at least 15 nucleotides in length, and may encode the entire amino acid sequence encoded by the *EcoRI/XhoI* fragment of clone 44B.1. The invention also includes a pair of nucleic acid primers comprising at least 10 contiguous nucleotides selected from or complementary to portion of a *EcoRI/XhoI* fragment isolated from bacteriophage clone 44B.1 deposited under ATCC accession No. PTA-3170 [N]. The primers of this invention will produce an amplified nucleic acid comprising at least 18 contiguous nucleotides of the *EcoRI/XhoI* fragment isolated from bacteriophage clone 44B.1 deposited under ATCC accession No. PTA-3170 [N]. The invention also provides replicons (e.g., nucleic acid vectors) comprising a sequence of at least 18 contiguous nucleotides selected from the sequence of a *EcoRI/XhoI* fragment isolated from bacteriophage clone 44B.1 deposited under ATCC accession No. PTA-3170 [N] or its complement under control of a promoter, as well as recombinant cells containing the replicon. *A 3*

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 5, lines 15-20:

-In another embodiment, this invention provides a substantially pure polypeptide comprising an amino acid sequence encoded by a *EcoRI/XhoI* fragment isolated from bacteriophage clone

44B.1 deposited under ATCC accession No. PTA-3170 — [N]; preferably, the polypeptide comprises at least one epitope, typically containing at least 9 amino acids. In a particular embodiment, the peptide of this invention consists essentially of an amino sequence encoded by said *EcoRI/XhoI* fragment. --

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 5, lines 21-26:

--In yet another embodiment, this invention provides an antibody which specifically binds to a mammalian protein comprising an amino acid sequence encoded by a *EcoRI/XhoI* fragment isolated from clone 44B.1 deposited under ATCC accession No. PTA-3170 — [N]. The antibody may be an isolated polyclonal antiserum, a preparation of purified polyclonal antibodies, or a preparation containing one or more monoclonal antibodies. --

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 5, line 27 extending to page 6, line 4:

A 4
--In still another embodiment, this invention provides a method for selecting variant nucleic acid sequences comprising (a) screening mammalian DNA or RNA with a nucleic acid probe comprising the nucleic acid of claim 1 or claim 2, (b) sequencing the DNA or RNA obtained in said screening, and (c) selecting DNA or RNA having sequences that differ from the nucleic acid sequence in a *EcoRI/XhoI* fragment of bacteriophage clone 44B.1 deposited under ATCC accession No. PTA-3170 — [N] by at least one nucleotide. --

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 6, lines 5-9:

A 5
--In yet another embodiment, this invention provides a method of screening for cancer in an individual comprising determining whether cells in the individual are expressing a gene product encoded by a *EcoRI/XhoI* fragment isolated from clone 44B.1 deposited under ATCC accession No. PTA-3170 — [N], expression of this product being correlated with an increased likelihood of cancer in the individual. --

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 7, lines 7-14:

A *b* *6*
-In yet another embodiment, this invention provides a method of screening for cancer in an individual comprising determining whether cells in the individual are simultaneously expressing two or more gene products selected from the group consisting of homeobox protein HOXA7, homeobox protein HOXB7, ADP-ribosylation factor 1 (Arf-1), ATP-dependent iron transporter ABC-7, and the protein encoded by a *EcoRI/XhoI* fragment of bacteriophage clone 44B.1 deposited under ATCC accession No. PTA-3170 [N], expression of a plurality of these gene products being correlated with an increased likelihood of cancer in the individual. *7*

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 8, lines 7-23:

A *7*
-In particularly preferred embodiment, this invention provides a method of screening for cancer in an individual comprising obtaining a sample of bodily fluid from the individual and determining whether or not the sample contains antibodies specific for one or more of the proteins selected from the group consisting of homeobox protein HOXA7, homeobox protein HOXB7, ADP-ribosylation factor 1 (Arf-1), ATP-dependent iron transporter ABC-7, and the protein encoded by a *EcoRI/XhoI* fragment of bacteriophage clone 44B.1 deposited under ATCC accession No. PTA-3170 [N], the presence of antibodies to any one of these proteins being correlated with an increased likelihood of cancer in the individual. Alternatively, the invention provides a method of screening for cancer in an individual comprising obtaining a sample of bodily fluid from the individual and determining whether or not the sample contains one or more proteins selected from the group consisting of homeobox protein HOXA7, homeobox protein HOXB7, ADP-ribosylation factor 1 (Arf-1), ATP-dependent iron transporter ABC-7, and the protein encoded by a *EcoRI/XhoI* fragment of bacteriophage clone 44B.1 deposited under ATCC accession No. PTA-3170 [N], the presence of any one of these proteins in the bodily fluid being correlated with an increased likelihood of cancer in the individual. *8*

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 8, line 24 extending to page 9, line 6:

A 8

--In yet another embodiment, this invention provides a method of cancer therapy comprising immunizing an individual with an immunogenic composition to elicit an immune response to one or more of the proteins selected from the group consisting of homeobox protein HOXA7, homeobox protein HOXB7, ADP-ribosylation factor 1 (Arf-1), ATP-dependent iron transporter ABC-7, and the protein encoded by a *EcoRI/XhoI* fragment of bacteriophage clone 44B.1 deposited under ATCC accession No. PTA-3170 [N]. Preferably, the immunogenic composition comprises at least one epitope of one or more of the proteins selected from the group consisting of homeobox protein HOXA7, homeobox protein HOXB7, ADP-ribosylation factor 1 (Arf-1), ATP-dependent iron transporter ABC-7, and the protein encoded by a *EcoRI/XhoI* fragment of bacteriophage clone 44B.1 deposited under ATCC accession No. PTA-3170 [N]. In a preferred mode, the immune response is a cellular immune response.

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 19, line 17 extending to page 20, line 3:

A ↑

--The nucleic acid probes described herein for use in screening gene libraries and selecting clones may also be used to detect mRNA transcripts in tumor cells that express the novel antigen of this invention. These probes preferably correspond to a sequence which encodes portions of the distinct sequences of the novel antigen (as deposited under ATCC accession No. PTA-3170 [N]). The probe can be either single or double stranded DNA or RNA. The size of a probe can vary from less than approximately 20 nucleotides to hundreds of nucleotides. The most desirable nucleotide probes do not detect nucleotide sequences unrelated to their intended target, do not show significant homology with unrelated nucleotide sequences, and do not contain complementary sequences such that they would self-hybridize or fold upon themselves. The guanine and cytosine content of desirable probes is not so high as to promote non-specific hybridization with unrelated sequences rich in guanine and cytosine. Finally, the melting temperature and free energy of binding are generally favorably suited to the detection technique for which they are intended. The probe may be radio-labeled, labeled with a fluorescent material, a biotinylated nucleotide, or the like. Procedures for the preparation and labeling of nucleotide probes are well known in the art.

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 22, lines 17-25:

As described below in Example 4, the inventors have observed autologous antibodies to five specific intracellular antigens in the serum of patients with ovarian cancer: HOXA7, HOXB7, ABC-7 (ATP-binding iron transporter), ADP-ribosylation factor, and the novel protein associated with the gene sequence in clone 44B.1, deposited under ATCC accession No. PTA-3170 [N]. This deposit has been made in accordance with the Budapest Treaty at the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Virginia 20110-2209, USA (www.atcc.org), on March 13, 2001. The inventors have also identified a previously unreported polymorph of HOXB7, which is detailed in Figure 12 (SEQ ID NO:1).
A 10

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 33, lines 24-27:

The cDNA were excised from recombinant λ ZAP bacteriophage by co-infection of *E. coli* strain XL1-Blue with Exassist~~EXASSIST~~ Interference resistant helper phage (Stratagene~~STRATAGENE~~). In this way the cDNA are transferred 3' to both *E. coli* and mammalian promoters in the phagemid vector pBK-CMV in *E. coli* XLOR.
A 11

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 34, lines 3-8:

DNA preparations of clones have been sequenced. The sequences were compared to known sequences in Genbank using BLAST searches. Five independent species have been identified among the 31 cDNA clones sequenced to date as summarized in Table 1. The novel sequence mentioned in Table 1 occurs as a fragment released by *EcoRI/XhoI* digestion of Clone 44B.1, deposited under ATCC accession No. PTA-3170 [N].
A 12

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 39, lines 6-27:

HOXB7 expression was determined by semi-quantitative RT-PCR in normal OSE and in IOSE-29 cells, a non-tumorigenic cell line established by immortalizing normal OSE cells with
A 13

SV40 large T antigen (Maines-Bandiera, S.L., Kruk, P.A. and Auersperg, N., *Am. J. Obstet. Gynecol.*, **167**:729-735, 1992). Reverse transcription was performed using 1 μ g of DNase I-treated total RNA, 500 ng of oligo(dT) and Superscript II reverse transcriptase (~~Life Technologies~~**LIFE TECHNOLOGIES**). Amplification of cDNAs for HOXB7 and for β -actin were performed as described by others (Alami, Y., Castronovo, V., Belotti, D., Flagiello, D. and Clausse, N., *Biochem. Biophys. Res. Comm.*, **257**:738-745, 1999; Nakajima-Iijima, S., Hamada, H., Reddy, P. and Kakunaga, T., *Proc. Natl. Acad. Sci. USA*, **82**:6133-6137, 1985) using Platinum Taq DNA polymerase (~~Life Technologies~~**LIFE TECHNOLOGIES**). Briefly, amplification was performed with a 2 min start at 94°C, denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, for 35 cycles for HOXB7 and 25 cycles for β -actin. Titrations were performed to ensure a linear range of amplification. Primers were the same as used by others (Alami, et al., 1999; and Nakajima-Iijima, et al., 1985) and were as follows: for HOXB7 5' AGAGTAACCTCCGGATCTA-3' (SEQ ID NO: 4) and 5'-TCTGCTTCAGCCCTGTCTT-3' (SEQ ID NO: 5), and for β -actin 5'-ATGATATCGCCGCGCTCG-3' (SEQ ID NO: 6) and 5'-CGCTCGGTGAGGATCTTCA-3' (SEQ ID NO: 7). Southern blot analysis of RT-PCR products was conducted using 32 P-labelled β -actin cDNA (~~Clontech~~**CLONTECH**, Palo Alto, CA) and HOXB7 cDNA. Hybridization signals were quantified by ~~PhosphorImager~~**PHOSPHORIMAGER** analysis (~~Molecular Dynamics~~**MOLECULAR DYNAMICS**, Sunnyvale, CA).--

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 45, lines 2-14:

14
- Since HOXB7 expression was markedly higher in carcinomas than in normal OSE and HOXB7 regulates proliferation of several other cell types (25-27), the possibility that HOXB7 over-expression increases proliferation of OSE cells was investigated. IOSE-29 cells were stably transfected with HOXB7 cloned in three different expression vectors. Full-length HOXB7 cDNA was subcloned from pPROEXHTb-HOXB7 into mammalian expression vectors pBK-CMV (~~Stratagene~~**STRATAGENE**) and pIRESpuro2 (~~Clontech~~**CLONTECH**). In addition, full-length HOXB7 cDNA, cloned in pcDNA3(17), was subcloned into pcDNA3.1 (~~Invitrogen~~**INVITROGEN**, Carlsbad, CA). Sub-confluent cultures of IOSE-29 cells were transfected with linearized DNA using Lipofectamine PLUS reagent (~~Life Technologies~~**LIFE**

TECHNOLOGIES). Cells transfected with pBK-CMV and pcDNA3.1 constructs were selected with G148 (400 μ g/ml) and cells transfected with pIRESpuro2 constructs were selected with puromycin (1 μ g/ml). Experiments were performed using lines established from single colonies.

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 45, line 24 extending to page 46, line 19:

A 15
Stably transfected IOSE29 cells were seeded at 2000 cells/200 μ l per well in 96-well plates. Total numbers of cells in each uncoated well were counted daily. Thymidine incorporation was measured in cultures pulsed for 3 h with 1 μ Ci of 3 H-methyl-thymidine (60Ci/mmol) (ICN, Costa Mesa, CA) following 1, 2, 3 and 4 days of culture. Shown in Figure 10 are the mean values of 3-4 independent experiments. Differences in cell numbers and thymidine incorporation levels of HOXB7-transfected cells, as compared with corresponding vector-transfected cells, at each time point were found to be statistically significant ($p<0.001$). The dramatically enhanced growth of HOXB7 transfectants was evidenced by increases in absolute cell number (Figure 10A) and in thymidine incorporation (Figure 10B), which were 3 to 4-fold the levels observed in vector-transfected cells. Cells were also seeded in wells coated with poly 2-hydroxyethylmethacrylate (poly(HEMA)) (SigmaSIGMA, St. Louis, MO) and pulsed for 18 h with 3 H-methyl-thymidine. Equivalent numbers of cells of vector- and HOXB7- transfected lines were seeded in wells coated with poly(HEMA) and proliferative activities monitored by measuring thymidine incorporation. Levels of incorporated thymidine in vector-transfected cells progressively declined, reaching almost background levels by Day 4 (Figure 10C). A similar rate of decline in thymidine incorporation in HOXB7 transfectants was observed (~50% decrease in levels per day), although levels of incorporated thymidine in HOXB7-transflectants were consistently higher than levels in vector-transflectants on any given day (Figure 10C). An initial increase in numbers of HOXB7-transfected cells during the first 24 h after seeding in poly(HEMA)-coated wells could explain their higher levels of thymidine incorporation, but HOXB7 over-expression in these cells does not appear to permit sustained anchorage-independent growth.

Please insert the following replacement paragraph for the paragraph currently found in the specification as originally filed on page 46, line 22 extending to page 47, line 3:

A 16

-- Growth factor autocrine loops represent a key mechanism regulating tumor cell growth. bFGF has been found by several studies to be expressed in ovarian carcinomas and is widely believed to stimulate their growth (28-30). It was therefore investigated whether over-expression of HOXB7 in OSE cells could up-regulate bFGF production. bFGF levels in culture supernatants collected from equivalent numbers of vector- and HOXB7- transfected IOSE-29 cells and lysates of these cells were assessed by ELISA. Cells were seeded in 25 cm² flasks containing 5 ml medium. Culture supernatants were harvested once cells reached a density of 2x10⁴ cells/cm². Protein lysates were prepared using M-PER reagent (PiercePIERCE) at 10⁵ cells/10 µl. bFGF levels were assayed in culture supernatants and cell lysates using the QuantikineQUANTIKINE human bFGF immunoassay (R&D Systems, Minneapolis, MN). --